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14. ABSTRACT:
Interleukin-2 (IL-2) has the capacity to activate lymphocytes to kill multi-drug resistant cancer cells. Our phase I data established the feasibility of administering a single course of low-dose IL-2 (1.6 million 1U/m2/day as a continuous i.v. infusion for 18 days) as consolidation treatment to patients with metastatic breast cancer early after intensive chemotherapy. We are performing a phase I1 trial of AC+T chemotherapy followed by IL-2 consolidation (1 cycle as described above) in high risk stage I1 and 111 breast cancer patients. Disease free survival and toxicity assessment represent major clinical aims (Specific aim 1). Immunologic effectors mechanisms induced following MAT/SR by IL-2 infusion are being evaluated (Aim 2). This study opened 6/11/03. Twelve patients been have accrued, 11 have completed planned treatment, 3 others are being screened. Two additional institutional sites are being added. Toxicity has been minimal to none. Two additional patients are being- screened for enrollment. Laboratory correlation studies are proceeding Our plan is complete accrual within 2 years, carrying over funds from preceding years.

15. SUBJECT TERMS
Breast Cancer, IL-2, Imrnunotherapy, Cytotoxicity, Drug Resistance

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Table of Contents

Introduction:

At least 46,000 women die from metastatic breast cancer each year in the United States[1,2]. Median survival remains 12-18 months from the diagnosis of metastatic disease, and progression-free survival beyond 5 years is rare (<10%)[3]. This has led to the testing of intensive chemotherapy, including marrow ablative therapy and stem cell rescue (MATISCR). Although this approach produced a high frequency of objective responses in patients with metastatic breast cancer, with up to 40-50% complete responses, responses tend to be short-lived. Only a minority of women (10-20%) achieved long-term disease free survival. Relapses may have be due to both minimal tumor contamination of stem cells reinfused into patients, as well as residual chemotherapy resistant tumor cells not cleared by the MATISCR regimen. IL-2 activated lymphocytes, termed lymphokine-activated killer (LAK) cells have significant cytotoxic activity against autologous breast cancer cells and breast cancer cell lines. Our own studies have demonstrated that multidrug-resistant tumor cells remain sensitive to LAK cell mediated killing.

We performed a phase I trial to test the feasibility of administering a single course of low-dose IL-2 (1.6 million IU/m^2/day as a continuous i.v. infusion) as consolidation treatment to patients with metastatic breast cancer early after MATISCR, This study established that IL-2 consolidation could be safely begun starting on day +14 post MATISCR with minimal toxicity. Substantial LAK cell induction was observed, using flow cytometric and cytotoxicity assays. Thus far, only 3 of 10 patients have had breast cancer relapse or progression, and a small second breast cancer was detected in 1 patient, Seven patients (60%) remain in complete remission at a median of 435 days (range: 224 - 720 days) post stem cell transplantation.

Patients with locoregional breast cancer with adverse characteristics, such as tumor size > 5cm with 1-3 positive lymph nodes, any tumor size and ≥4 lymph nodes involved, tumor invading through lymph node capsule, involvement of internal mammary lymph nodes, chest wall or skin infiltration and inflammatory characteristics also have a poor prognosis. For example, women with 4-10 lymph nodes involved have an approximately 40% 10 year disease-free survival (DFS), while women with >10 lymph nodes positive have an approximately 20% 10 year DFS[1,4]. Patients with Stage IIIA and IIIB breast cancer have 5 year survivals of 40-50%[1,5]. Survival with inflammatory breast cancer with aggressive multi-modality therapy is estimated to be 50% at 5 years and 35% at 10 years[1]. Methods to improve these results, incorporating novel therapeutics, are of significant importance for the healthcare of these patients,

In this application we proposed to perform cytoreduction in patients with metastatic breast cancer using doxorubicin/cyclophosphamide plus taxane chemotherapy, followed by an 18 day continuous infusion of low-dose IL-2 starting on day +14 to activate lymphocytes to kill residual chemotherapy-resistant cancer cells (the grant objectives were revised in 2003). Based on preliminary data, we hypothesize that a single course of IL-2 will result in a significant improvement in disease-free survival, with minimal toxicity. Effectiveness of this approach may correlate with the effective induction of LAK precursor and effector cells, as well as evidence for reduction in the burden of minimal residual cancer cells. In **Specific Aim 1** we will perform a prospective Phase II clinical trial to test whether the addition of 1 cycle of continuous i.v. infusion of IL-2 in women with metastatic breast cancer, starting on day +14 after treatment with AC+T

chemotherapy, to assess toxicity and to estimate progression-free and overall survival: In **Specific Aim** 2 we will evaluate possible immunologic effector mechanisms induced following MATISCR and IL-2 infusion. Phenotypic and functional assays for LAK cell induction and enzyme immunoassays for circulating pro-inflammatory cytokines will be performed.

Body:

Initiation of our initial clinical trial proposed was delayed by a number of unanticipated events. First, shortly after this proposal was funded in 1999, a series of randomized trials was reported at the American Society of Clinical Oncology meetings in 5/00 comparing standard dose chemotherapy and marrow ablative therapy and stem cell rescue (MATISCR) for treatment of advanced breast cancer. The conclusions of all but one of these trials was that there was no advantage to stem cell transplants in breast cancer patients over standard chemotherapy[6-8](1-3). The second event was that the one trial showing benefit of MATISCR over chemotherapy (Bezwoda, et al) was found to contain fraudulent data[9]. In combination, these findings made our clinical trial including MATISCR unacceptable. Since the goal of MATISCR in our trial was to provide maximal cytoreduction prior to IL-2 based immunotherapy, this goal was still felt scientifically reasonable, given our impressive phase I trial results. In order to further prove the validity of these observations, we felt that a change from a randomized trial to a single arm phase II study (MATISCR followed by an 18 day infusion of IL-2) was warranted. This change was discussed with the USAMRMC and the study protocol and consent documents were rewritten. A third point holding up the clinical trials was due to negotiations between the University of Utah lawyers and the USAMRMC concerning required liability clauses in the consent document and final approval by the University of Utah IRB. After many months of negotiations, a finalized consent language and protocol was agreed upon. A final draft has been submitted to the University of Utah IRB and was approved with minor revisions. Further investigation into fraudulent data published by Bezwoda was presented at the ASCO meetings in 5/01. This resulted in the general abandonment of MATISCR as a breast cancer treatment modality in the United States. At the end of August of 2001, I was notified by my co-investigator Dr. Peterson that we would not be able to accrue any patients to a MATISCR regimen based trial, since patient referral to the University of Utah BMT program had virtually stopped.

We therefore reevaluated our options. Due to our exciting preliminary results, we still strongly believe that the concept of IL-2 consolidation in high-risk breast cancer should be tested after maximal cytoreduction. Given the apparent equivalence of MATISCR and standard chemotherapy in high risk breast cancer patients, we concluded that an alternative method to test our hypothesis is to enroll high-risk breast cancer patients who are treated systematically with surgery, followed by a standard chemotherapy regimen (doxorubicin/cyclophosphamide followed by paclitaxel or docetaxel)[10,11], followed by a 18 day infusion of IL-2. Patients will subsequently receive irradiation to the breast and regional node areas. Patients deemed at high risk include: patients with $\geq$4 lymph nodes positive (40% 5 year survival with >4+ nodes, <20% 5 year survival with >10 nodes involved), inflammatory breast cancer (<20% 5 year survival) and patients with resected stage IV disease (Stage IV NED, <10-20% 5 year survival)[12].

Two breast cancer medical oncology specialists (Dr. John H. Ward and Dr. Saundra

Buys), from the Huntsman Cancer Institute were added as co-investigators to ensure adequate accrual (to replace Dr. Petersen, a MAT/SCR specialist). We also obtained a commitment from Chiron to provide recombinant IL-2 for this trial. The protocol finally opened to patient accrual 6/11/03 at the Huntsman Cancer Institute. Twelve patients have been enrolled, and 11 have completed planned therapy. Toxicity to date has been minimal to none (no toxicity > grade 1 noted). One patient had a brief interruption in treatment due to fever, which subsided and the patient was able to complete planned treatment. Two patients have experienced a cancer relapse (this appears lower than expected in this high risk population). Three additional patients are currently being screened for enrollment. Clinical samples have been submitted to the laboratory for testing on all 11 patients. Preliminary results indicate induction of circulating LAK cells in all patients, albeit with variability in maximum cytotoxicity at 100:1 effector to target cell ratio. It will be interesting to see if increased cytotoxicity correlates with eventual treatment outcome and progression-free survival.

Due to low accrual of this relatively infrequent breast cancer population, we are expanding our institutional participation to include Mountain States Tumor Institute (St. Lukes Hospital) in Boise and Big Sky Oncology, Great Falls Montana. These large multi-oncologist groups see large numbers of breast cancer patients and are expected to increase the rate of trial accrual to allow completion of the phase II trial. The paperwork and contracts needed for this collaboration has taken over 9 months to arrange, and is just about finalized. It is envisioned that patient accrual for the proposed phase II trial (16 additional patients) can be completed in 24 months. The Huntsman Cancer Institute Clinical Trials Office has committed to provide the resources for the long-term follow-up patients beyond the scope of the current grant funding. The revised protocol has been approved by the University of Utah IRB and by the US Army Medical Research and Material Command (HSRRB Log Number A-9034). Our proposal is to complete patient accrual over 24 months, as a no-cost extension, using funds carried over from preceding years (with additional support from the Huntsman Cancer Institute).

Key research accomplishments:

We treated 20 patients with MAT/SCR in our phase I pilot trial. Patients received IL-2 either starting on day 1 (10 patients) or day 14 (10 patients), following stem cell infusion. A total of 17 patients were evaluable for response at the time of initial analysis. A total of 17 patients (85%) completed the IL-2 course. Three patients receiving IL-2 from day 1 required IL-2 infusions to be terminated early (2 fever, 1 thrombocytopenia). Relapse free survival was 45% with 580 day median follow-up (135-1175 days), with 75% overall survival.

LAK cell activation was evaluated in patients undergoing IL-2 infusions starting either day 1 (5 patients) or day 14 post stem cell infusion (5 patients). Cytotoxicity against the MCF-7 breast cancer line was detected in all patients, regardless of whether the IL-2 infusion was started day 1 or 14. Increased cytolytic activity was detected in cytotoxicity assays performed with the addition of IL-2, suggesting a substantial increase in circulating LAK cell precursors in both patient populations. Phenotypic evaluation established that while CD56+ cell populations were expanded in both patient groups, the absolute number of circulating CD56+ cells was 10-fold higher in patients receiving IL-2 starting on day 14.

Due to these results, our current clinical trial will treat patients beginning on day +14 after completion of 4 cycles of AC and paclitaxel or docetaxel with an 18-day infusion of IL-2 to verify these exciting clinical results in this high-risk breast cancer population. Twelve patients have been entered to date. Treatment related toxicity has been negligible (none > grade 1). There has been no disease progression to date (follow up too short).

Reportable outcomes:

Abstract presented at Era of Hope Meeting 9125102-9128102 (enclosed)

Petersen FB, **Samlowski** WE. Feasibility of low dose continuous infusion of IL-2as a consolidation treatment following intensive breast cancer chemotherapy. Proceedings Era of Hope DOD Breast Cancer Research Program Mtg. 2002, p 33-20

Conclusions:

The proposed use of IL-2 following maximal cytoreduction of tumor by standard chemotherapy or MATISCR remains promising based on our preliminary data, with 45% of patients achieving >2 year disease free survival after MATISCR plus IL-2 and 9 of 11 patients disease free on the current trial with up to 2 year follow-up. We are continuing a phase II pilot study to evaluate the effectiveness of this regimen with standard adjuvant chemotherapy in high-risk breast cancer patients. Preliminary data shows toxicity to be minimal and feasibility of immunologic monitoring.

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